

## PREY PREFERENCE IN *GENLISEA* SMALL CRUSTACEANS, NOT PROTOZOA

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Keywords: cultivation: carnivory: amphipods, copepods, protozoans, *Genlisea*.

### Introduction

It has been reported that *Genlisea* (see Back Cover) is a plant which specializes in trapping ciliate protozoa, such as *Blepharisma*, which it has chemotactically attracted, and it was claimed further to be the first such example known (Barthlott *et al.* 1998). Those authors used both South American (*G. aurea*, *G. violacea*) and African (*G. margaretae*) species for their work, and they found or were able to cite evidence for prey being attracted by means of released chemicals, for the release of digestive enzymes, and the absorption and translocation of radioactive <sup>35</sup>S (Barthlott *et al.* 1998).

However, a number of questions have been raised about this claim (Plachno *et al.* 2005a). First, the prey offered to the plants in the experiments described above may be very different from the prey which these plants would encounter in nature, making the identification of *Genlisea* as specializing in trapping protozoa suspect. Naturally occurring organisms which might serve as prey might also include small crustaceans and other small animals such as water bears.

Second, while the mechanism of the trap was at one point proposed to involve flow of water, and which might be ideal for capture of protozoa (Meyers-Rice 1994), it was subsequently found to have no water flow involved (Adamec 2003). Instead, the mechanism seems to involve both inward-pointing hairs and mucilage (Stuidnicka 2003a, 2003b) which help to point potential prey into the digestive bulb of the trap and to prevent their escape. Given the presence of the mucilage and hairs, these traps may be difficult for small protozoa to enter and may be adapted more for catching larger, stronger, more nutrient-rich prey like small crustaceans which are better equipped to enter the traps.

To test the prey spectrum of *Genlisea*, experiments were performed using *G. filiformis* from South America; 1) to determine which types of prey organisms were preferentially trapped when a mixture of prey was presented to the plants and 2) to determine whether the released chemoattractants affected ciliate protozoa, as suggested by Barthlott *et al.* (1998).

### Materials and Methods

*Genlisea filiformis* plants from old tissue cultures, originally made on 1/5-strength tissue culture media (Darnowski 2004), low in nutrients were used, after hardening off on soil (sand-peat mixture). This ensured that plants were not well-stocked with nutrients and thus prevented high nutrient levels from reducing the drive for carnivory.

For the first type of experiment, repeated thrice, plants were placed in a dish with their white trapping leaves in filtered pond water. Then, potential prey were added as follows: 3 drops of a culture of *Blepharisma* sp., a ciliate protozoan used by Barthlott *et al.* (1998; provided hundreds of organisms); 3 drops of a culture of *Euglena acus*, a flagellate protozoan (hundreds of prey provided); 4 drops of a culture of *Hypsibius* sp., a waterbear (animal phylum, size of a typical 10µm protozoan; dozens to hundreds provided); 3 drops of a culture of copepods (*Cyclops* sp.), a small crustacean (about 10 provided); 3 drops of a culture of amphipods (*Gammarus* sp.), a small crustacean about 10 times larger than *Cyclops* (about 5 provided). Organisms were left with the plants for 1 week, and then trapping was scored using a dissecting microscope.

For the second type of experiment, bactoagar cubes approximately 0.5 cm (0.2 in) on a side were placed either in pond water (control) or under the trapping leaves of *G. filiformis* growing in soil for 1

week to allow absorption of chemicals from the local environments. Chemoattractants from *G. filiformis* should have been absorbed during this time by the agar cubes. These were then placed in the center of 100 mm plastic petri dishes in filtered pond water, and prey were added as noted above except for amphipods, since these were never trapped in the first type of experiment. After about a half-hour for equilibration and diffusion of chemoattractants, the position of prey of each type was found for about 5 individuals found by randomly selecting fields of view around the dish under a dissecting microscope.

Position was recorded relative to the agar cube. The mean distance from the block was determined for each prey item, and the experiment was repeated three times. Then, the overall mean and the standard error of the mean were calculated for each prey item across the three repetitions.

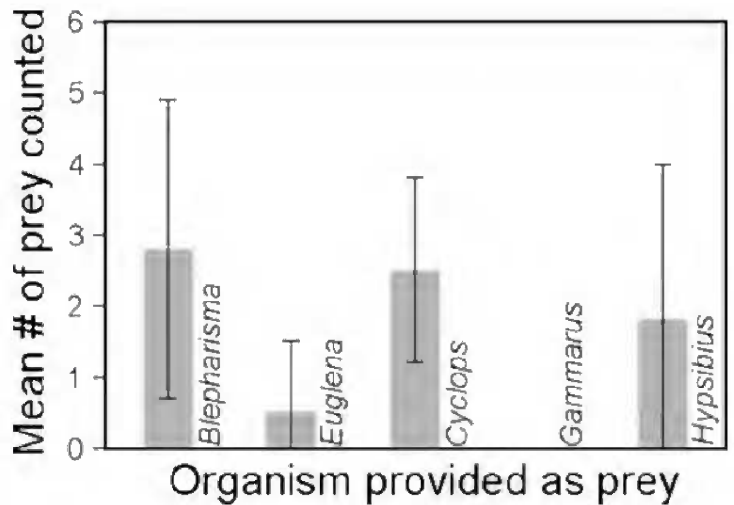


Figure 1: Prey counted in *Genlisea filiformis* traps. *Blepharisma*:  $2.8 \pm 2.1$ , *Euglena*:  $0.5 \pm 1$ , *Cyclops*:  $2.5 \pm 1.3$ , *Gammarus*: 0.0, *Hypsibius*:  $1.8 \pm 2.2$ .

### Results

As can be seen from Figure 1, the *G. filiformis* plants never trapped amphipods, and they trapped about the same number of copepods as other prey, all of the other prey being much smaller than the copepods.

Figure 2 shows the results of the experiment with chemoattractants absorbed by agar blocks. The data are graphed with 2.5 cm subtracted from each distance. This was done to emphasize whether position was random or not, since 2.5 cm is the average distance from the center of the dish to the edge. If prey were distributed away from the agar block in no particular position, a mean distance of 2.5 cm would be expected. Negative numbers indicate closer placement towards the agar block, demonstrating attraction by the block/chemicals diffusing from it, and positive numbers indicate movement away from the block, i.e. repulsion by released chemicals. The distance from the center of the dish at which a circle can be drawn to evenly divide the area of the dish into two parts is at about 3.6 cm, based on simple geometric considerations. However, given that the dish has a reflective edge at the outside of the dish but not at the center, and such an edge might well deter negatively phototactic prey, the experimenter has chosen the midpoint of the radius of the dish, 2.5 cm, instead. Even if the data were recentered around the 3.6 cm line, the basic conclusion of this paper would not be changed.

### Discussion

From the data presented, clearly prey preference in *Genlisea* is for small crustaceans, not protozoa. Figure 1 shows that *G. filiformis* trapped relatively few prey of any type, but the prey counted most frequently were the protozoan *Blepharisma* and the copepod *Cyclops*. However, the protozoan prey were presented with an abundance of approximately 10 times that of the copepods. Therefore, the plants trapped a much higher percentage of the copepods than any other type of prey. This tends to contradict the conclusions of Barthlott *et al.* (1998) that *Genlisea* specializes in trapping protozoa.

Further, from Figure 2, *Blepharisma* seems to be neither attracted nor repulsed by either control or *Genlisea*-blocks of agar. Other prey, including copepods, stayed further than 2.5 cm from the blocks for controls but came closer than 2.5 cm to the *Genlisea*-block. This may show that chemoat-

tractants had been absorbed by the blocks and that some prey were indeed attracted by them, including the flagellate protozoan *Euglena acus*.

Taken together, these two experiments suggest that *Genlisea* does emit water-soluble chemoattractants which do attract a range of prey, both protozoa and animals. Further, proportionally more copepods were trapped than protozoa, and each copepod contains far more resources than each protozoan. Thus, the conclusion of Barthlott *et al.* (1998) that *Genlisea* specializes in trapping protozoa is probably incorrect.

This study does not address issues of the differential stability of the remains of different prey. Certainly crustacean prey with their indigestible chitinous exoskeletons would leave longer-lasting traces than easily-digested protozoa and might be overrepresented in the data here. However, given that crustaceans are generally much larger than protozoa and provide much more nutrients per prey caught, the data shown here do contradict the previous assertion that *Genlisea* specializes in protozoa.

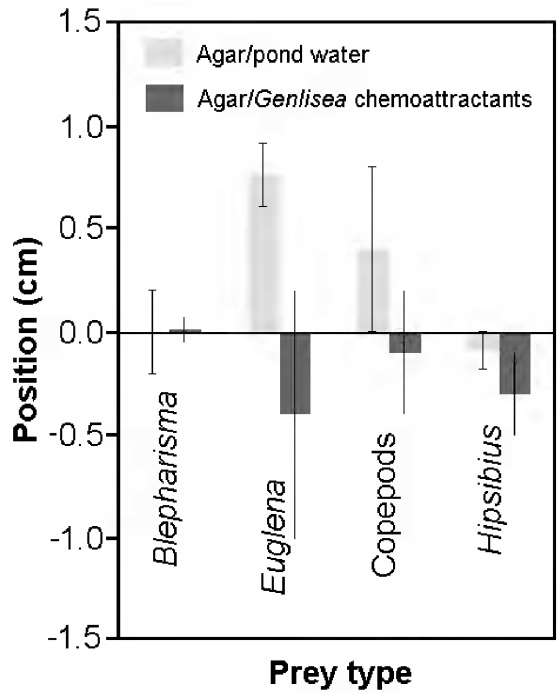


Figure 2: Distances of prey, minus 2.5 cm, from the *Genlisea* or agar block bait.

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